Leukaemia Diagnosis Interpretation
All Participants

Participant: 42981 **Trial No: 151605**

Date Issued: 28-March-2016 **Closing Date: 15-April-2016**

Trial Comments

This was an electronic only trial issued to a total of 769 participants.

Your Diagnosis: Follicular lymphoma

Consensus Diagnosis: Follicular lymphoma

Your Diagnosis is classed as a **CORRECT** result

Performance Score: 0

Running Score: 100

Consensus

	Consensus Diagnosis	Your Diagnosis
Lineage	Lymphoid-B cell	Lymphoid-B cell
Subclassification	Mature B-cell neoplasms	Mature B-cell neoplasms
Diagnosis	Follicular lymphoma	Follicular lymphoma

Breakdown of returned diagnoses

Diagnosis	No Of Returns	Percentage Of Returns
Total Returns	302	100.0
Follicular lymphoma	293	97.0
Diffuse large B-cell lymphoma (DLBCL), NOS	6	2.0
T-cell prolymphocytic leukaemia	1	0.3
Lymphocyte-rich classical Hodgkin lymphoma	1	0.3
Burkitt lymphoma	1	0.3

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Leukaemia Diagnostic Interpretation trial 151605

Case History

A 74 year old male presented with a lump in his neck. This had been present for the previous 5 weeks and had not changed in size during this time. The patient displayed no infective symptoms.

During examination, the patient was found to have bilateral lymphadenopathy, the largest measuring 3cm.

A CT scan showed enlarged nodes in the neck, chest, abdomen and pelvis. Splenomegaly measuring 21.4cm was seen.

Haematological examination revealed the following:

Hb- 132 g/L; WBC- 290.64 x10^9/L; PLT- 158 x10^9/L; RBC- 4.27 x10^12/L; HCT- 0.410 L/L; MCV- 96.0 fL; MCH- 30.9 pg, Neutrophils 17.83 x10^9/L, Lymphocytes 268.10 x10^9/L, Monocytes 4.07 x10^9/L

Immunophenotype

The consensus results of the malignant cells had the following phenotype (consensus percentage expression in brackets rounded to the nearest whole percentage).

Positive antigens – CD10 (28%), CD19 (67%), CD20 (95%), CD22 (47%), CD45 (100%), CD79a (70%), CD79b (73%), CD200 (40%), FMC7 (76%), HLADR (93%), Kappa (55%)

Negative antigens – CD2 (0%), CD3 (0%), CD5 (1%), CD13 (0%), CD23 (2%), CD34 (0%), CD38 (3%), CD43 (1%), Lambda (1%)

Cytogenetics and Molecular genetics

FISH analysis of 100 interphase nuclei showed an IgH:BCL2 fusion consistent with a t (14;18) (q32;q21) translocation

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Histology

A core biopsy was undertaken which showed 2 lymph node cores where the architecture was completely effaced. Mainly nodular proliferation of small to medium sized cells was seen. Frequent centroblasts present in some areas (>15/hpf (high power field)) and are admixed with centrocytes.

Immunohistochemistry

Lymphoid cells showed diffuse and strong staining with CD20, CD10, Bcl2, Bcl6, MUM1. The follicular dendritic cell meshworks which were focally expanded stained with CD23. A reactive T cell population stained with CD3 and CD5. EBV (EBER-ISH) was negative. There was probable kappa light chain restriction. The proliferation index (Ki67) was variable and on average was 30-40%.

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Peripheral Blood Morphology

Morphology comments from Professor Wendy Erber
Consultant Haematologist, University of Western Australia, Nedlands, Australia

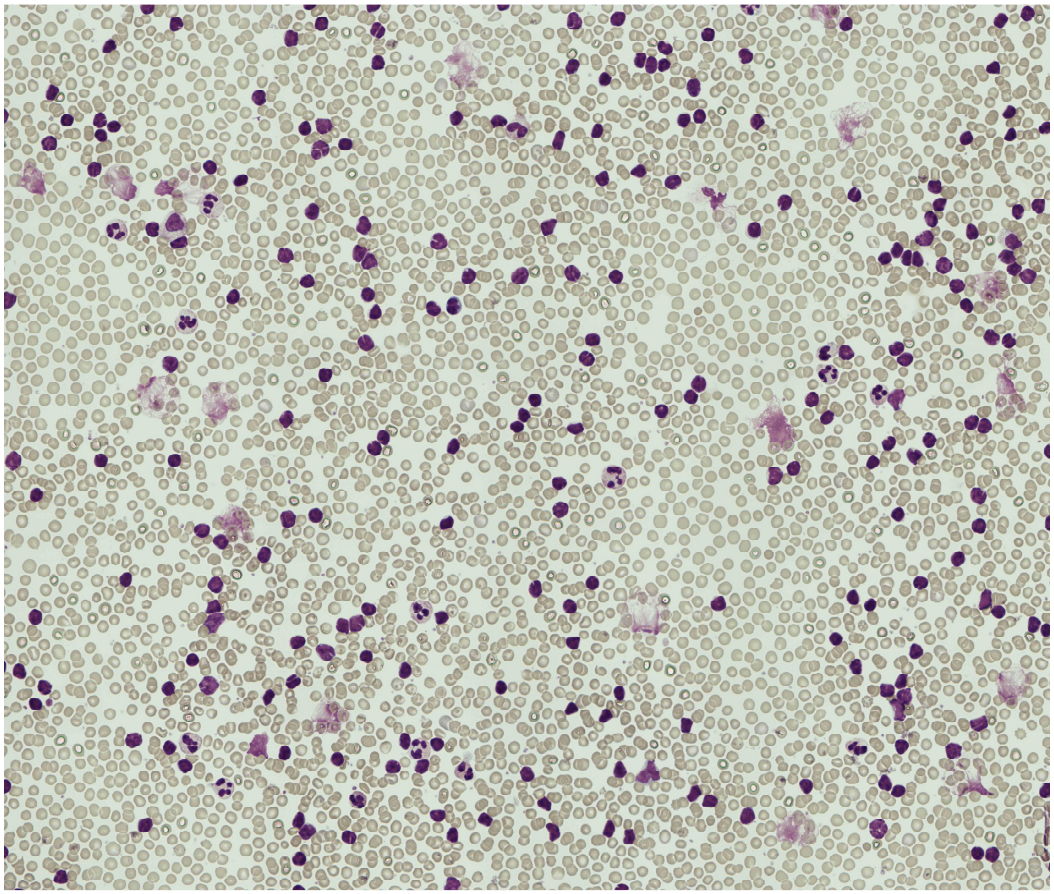
COMMENT:

The blood film shows a marked leucocytosis with the majority being small lymphocytes. The majority have minimal cytoplasm and an extremely high nuclear: cytoplasmic ratio. The nuclei of some cells are indented. The nuclear chromatin is mature ("featureless") and homogeneous. The cells do not have a nucleolus. The cells have minimal cytoplasm which is visible at the nuclear cleft. Erythrocytes, neutrophils and platelets appear normal. Smear cells are present.

Diagnosis: Leukaemic phase of follicular lymphoma.

Examples of Digital Images Issued

Figure 1: Peripheral Blood x50 magnification (May Grünwald Giesma stain)



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Exercise Conclusion / Case Discussion

Please note that, because this trial requires a diagnosis based on the WHO guidelines, this report is therefore based on the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition[2].

Leukaemia Immunophenotyping - Part one

There was a large SD on CD10 and CD22. This case was known to be positive for both these antigens but when the data was examined it can be clearly seen that those using PE (and to a lesser degree APC for CD22) detected the antigens. However, those using FITC conjugated CD10 and CD22 missed this antigen and therefore got the answer wrong. This could/will have potentially impacted upon arriving at the correct diagnosis when interpreting the results in the context of disease immunophenotype.

Those using CD79b FITC and CD79 PerCP-CY5.5 conjugated antigen also had an increased potential to miss the expression of this antigen. We found that those laboratories using CD79b conjugated with either FITC or PerCP-CY5.5 were over 40% more likely to miss this antigen compared to those using PE and APC. Up to 41% of laboratories using CD79b conjugated with FITC or PerCP-CY5.5 obtained a result of less than 30% (and 34% less than 20%), whereas those using PE or APC only 3.5% had a result less than 30% (and the same for less than 20%).

The findings from this trial, yet again, underline the importance of ensuring that when detecting antigens that could have weak expression the correct fluorochrome must be used to ensure detection.

This programme is due to be redesigned in the next 12-18 months and one aspect we will be examining the possibility of using results that are the “correct” result as the target value and thus penalising laboratories where the antigens have been missed, particularly due to inappropriate fluorochrome use. Thus, in the examples above where it is known that an antigen is positive and can be detected using the correct antibody/fluorochrome combination those using the wrong antibody/fluorochrome combination would have been penalised.

Leukaemia Diagnostic Interpretation - Part Two

Exercise Consensus Primary Diagnosis

(Classified as Correct by members of the UK NEQAS LI Steering Committee)

Follicular Lymphoma

Other Differential Diagnoses (Classified as Minor Errors):

Diffuse Large B-Cell Lymphoma (DLBCL), NOS

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Other Differential Diagnoses (Classified as Major Errors):

T-cell Prolymphocytic Leukaemia
Lymphocyte-Rich Classical Hodgkin Lymphoma
Burkitt Lymphoma

Rationale for Correct Classification

This case was referred to us from a participant for use in the programme.

Follicular lymphoma (FL) is the most common type of lymphoma seen in the Western World, accounting for approximately 20% of all lymphoma cases with the postulated normal counterpart being the germinal B-cell. Its incidence is lower in Eastern Europe and Asia. It is a disease associated with adults, rarely occurring in individuals below the age of 20. When it is occurs below the age of sixteen it is usually in males but in adults the male:female ratio is approximately 1:1.7 and occurs predominantly in the 60th decade.

Immunophenotypically this case was typical of a FL with weak expression of CD19 and CD10. Cytogentically there was a t14:18 IgH;BCL2 translocation (this occurs in 90% of grade 1-2 cases). It is worth noting that with BCL2 rearrangements become infrequent as the disease progresses. However, it is also worth noting that FL has 4 separate ICD-O codes due to the grading of FL. The grading of FL is actually a histological grading and based upon the number of centroblasts seen per high power field (hpf). Interestingly, this case was noted to have >15 centroblasts per hpf and the WHO classification states in such instances the case should be reported as diffuse large B-cell lymphoma with follicular lymphoma. Thus, in this instance it must be graded as a grade 3 and as centrocytes are present a grade 3A. Grade 3B is when the follicles are composed entirely of large blastic cells. However, it should also be noted that if, on histological examination, there are areas that are entirely centroblasts then a diagnosis of DLBCL must be made. There was no evidence of this as reported by the referring centre. Furthermore, DLBCL usually arises *de* novo but can arise from less aggressive lymphomas such as FL. In FL, BCL6 and MUM1 is typically absent but in this case there was some histochemical diffuse staining that is in keeping with a higher grading. The expression of Ki-67 was reported to be between 30-40% and in DLBCL is usually >40% and quite often greater than 90%.

Thus, on balance, the Steering Committee concluded that **the primary diagnosis** was a Follicular Lymphoma (probably a grade 3A).

A minor error was, for the purpose of this trial, judged to be Diffuse Large B-Cell Lymphoma based on the evidence presented above.

Those diagnoses submitted that were considered incorrect were: T-cell prolymphocytic leukaemia (this case lacks any T-cell antigens); Lymphocyte-rich classical Hodgkin lymphoma, this disease is primarily nodular and has polyclonal

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lymphocytes and not seen in the peripheral blood; and, Burkitt lymphoma because the morphology and immunophenotype was not in keeping with this disease

It should be noted that when submitting a diagnosis it is **the primary** diagnosis that should always be submitted, not any differential diagnosis that may be considered. It is also worth highlighting that all our programmes are undergoing major overhaul and this programme will be completely overhauled in the next 12-18 months.

Further Trial Findings

Table 1: Submissions from all participants - laboratories and individuals.

Diagnosis	Total No. of Returns	No. of Laboratories	No. of Individual
Follicular lymphoma	293	202	91
Diffuse large B-cell lymphoma (DLBCL), NOS	6	6	0
T-cell prolymphocytic leukaemia	1	0	1
Lymphocyte-rich classical Hodgkin lymphoma	1	0	1
Burkitt lymphoma	1	0	1

Table 2: Submissions from laboratory participants only

Diagnosis	Laboratory Returns %
Follicular lymphoma	97.1
Diffuse large B-cell lymphoma (DLBCL), NOS	2.9

Table 3: Submissions from individual participants only

Diagnosis	Individual Returns %
Follicular lymphoma	96.8
T-cell prolymphocytic leukaemia	1.1
Lymphocyte-rich classical Hodgkin lymphoma	1.1
Burkitt lymphoma	1.1

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References:

1. Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition. IARC Press 2008

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
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4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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